(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 May 2004 (21.05.2004)

PCT

(10) International Publication Number
WO 2004/041251 A1

(51) International Patent Classification[7]: A61K 9/16, A23L 1/22

(21) International Application Number: PCT/CA2003/001699

(22) International Filing Date: 4 November 2003 (04.11.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/423,363 4 November 2002 (04.11.2002) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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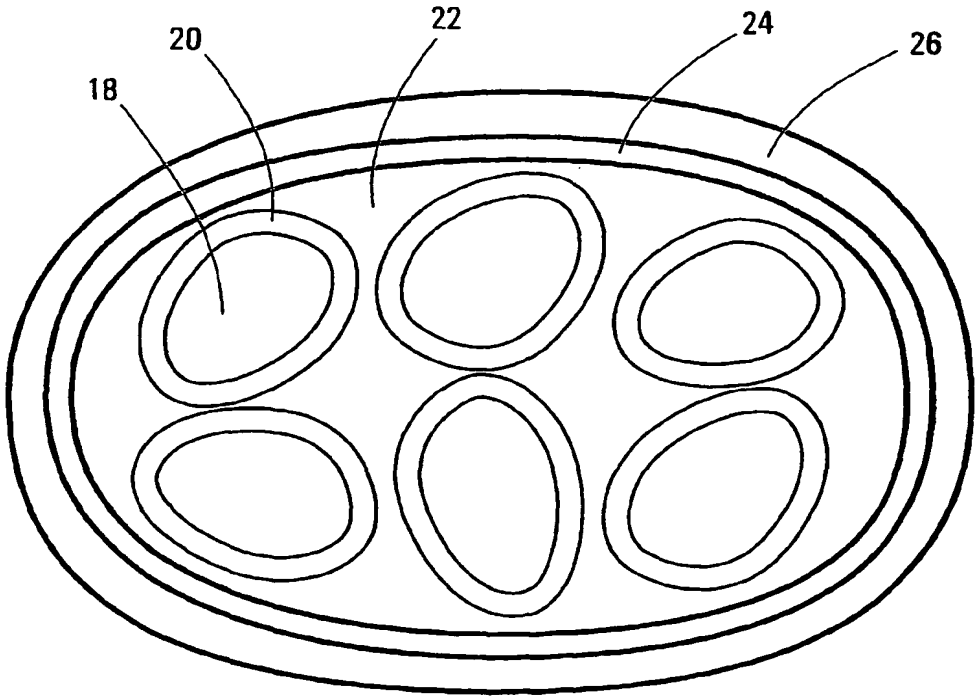
(54) Title: MICROCAPSULES HAVING MULTIPLE SHELLS AND METHOD FOR THE PREPARATION THEREOF




(57) Abstract: Single-core and multi-core microcapsules are provided, having multiple shells, at least one of which is formed of a complex a coacervate of two components of shell materials. The complex coacervate may be the same or different for each shell. Also provided are methods for making the microcapsules.

WO 2004/041251 A1

**Published:**

— *with international search report*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

# MICROCAPSULES HAVING MULTIPLE SHELLS AND METHOD FOR THE PREPARATION THEREOF

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of United States Provisional Patent Application No. 60/423,363 filed November 4, 2002, which is incorporated by reference herein in its entirety.

## FIELD OF THE INVENTION

This invention relates to microcapsules having multiple shells, to methods of preparing microcapsules and to their use.

## BACKGROUND OF THE INVENTION

Microcapsules are small particles of solids, or droplets of liquids, inside a thin coating of a shell material such as starch, gelatine, lipids, polysaccharides, wax or polyacrylic acids. They are used, for example, to prepare liquids as free-flowing powders or compressed solids, to separate reactive materials, to reduce toxicity, to protect against oxidation and/or to control the rate of release of a substance such as an enzyme, flavour, a nutrient, a drug, etc.

Ideally, a microcapsule would have good mechanical strength (e.g. resistance to rupture) and the microcapsule shell would provide a good barrier to oxidation, etc.

A typical approach to meeting these requirements is to increase the thickness of the microcapsule wall. But this results in an undesirable reduction in the loading capacity of the microcapsule. That is, the "payload" of the microcapsule, being the mass of the loading substance encapsulated in the microcapsule divided by the total mass

of the microcapsule, is low. The typical payload of such "single-core" microcapsules made by spray drying an emulsion is in the range of about 25-50%.

Another approach to the problem has been to create what are known as "multi-core" microcapsules. These microcapsules are usually formed by spray drying an emulsion of core material such that the shell material coats individual particles of core material, which then aggregate and form a cluster. A typical multi-core microcapsule is depicted in prior art Figure 1. Multi-core microcapsule 10 contains a plurality of cores 12. The cores 12 take the form of entrapped particles of solids or of liquid droplets dispersed throughout a relatively continuous matrix of shell material 14. As a result, there is a high ratio of shell material to loading material and the payload of the multi-core microcapsule is therefore low. Moreover, despite the high ratio of shell material to loading substance in such microcapsules, the shell material is poorly distributed. As shown in prior art Figure 1, many of the cores 12 are very close to the surface 16 of the microcapsule. The cores at the surface are therefore not well protected against rupture or from oxidation.

Known microcapsules therefore either have a poor payload, or fail to adequately contain and protect the loading substance deposited therein. Moreover, because these microcapsules are generally prepared in a single step, it is difficult to incorporate multiple functionalities, such as oxidation resistance, moisture resistance and taste masking into a single microcapsule.

## SUMMARY OF THE INVENTION

In one aspect, the invention provides a multi-core microcapsule comprising: (a) an agglomeration of primary

microcapsules, each primary microcapsule comprising a core and a first shell surrounding the core; (b) a second shell surrounding the agglomeration; and (c) a third shell surrounding the second shell; at least one of the first, second and third shells comprising a complex coacervate.

In another aspect, the invention provides a single-core microcapsule comprising: (a) a core; (b) a first shell surrounding the core; and (c) a second shell surrounding the first shell; at least one of the first and second shells comprising a complex coacervate.

In the case of either the multi-core or single-core microcapsules, it is preferred that all of the shells comprise a complex coacervate, which may be the same or different for each of the shells. Additional shells, e.g. from 1 to 20, may be added to further strengthen the microcapsule.

In another aspect, the invention provides a process for making a microcapsule having a plurality of shells, the process comprising:

(a) providing a microcapsule selected from the group consisting of:

(i) a multi-core microcapsule comprising: an agglomeration of primary microcapsules, each primary microcapsule comprising a core and a first shell surrounding the core; and a second shell surrounding said agglomeration; and

(ii) a single-core microcapsule comprising: a core; and a first shell surrounding the core;

(b) mixing the microcapsule with first and second polymer components of shell material in aqueous solution;

(c) adjusting at least one of pH, temperature, concentration and mixing speed to form shell material comprising the first and second polymer components, the shell material forming an additional shell enveloping the microcapsule;

wherein at least one of the first shell, the second shell and the additional shell comprises a complex coacervate.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts a typical prior art multi-core microcapsule.

Figures 2 and 3 depict embodiments of the invention in which multi-core microcapsules are provided having multiple shells.

Figures 4 and 5 depict embodiments of the invention in which single-core microcapsules are provided having multiple shells.

Figure 6 is a photomicrograph of multi-core microcapsules prepared with a one-step process (62% payload), prepared for purposes of comparison.

Figure 7 is a photomicrograph of multi-core microcapsules prepared with a two-step process in accordance with the invention (59% payload).

Figure 8 is a photomicrograph of multi-core microcapsules prepared with a two-step process in accordance with the invention in which alginate is incorporated in the outer shell (53% payload).

Figure 9 is a photomicrograph of multi-core microcapsules prepared with a three-step process in which

lipids and alginate are incorporated in an inner shell while gelatine and polyphosphate forms an outer shell.

Figure 10 is a photomicrograph of multi-core microcapsules prepared with a two-step process in which lipids and alginate are incorporated in the second shell.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

### Core Materials

Any core material that may be encapsulated in microcapsules is useful in the invention. Indeed, in certain embodiments, commercially available microcapsules may be obtained and then further processed according to the processes of the invention.

When the initial multi-core microcapsules are prepared according to processes as described herein involving an aqueous solution, the core material may be virtually any substance that is not entirely soluble in the aqueous solution. Preferably, the core is a solid, a hydrophobic liquid, or a mixture of a solid and a hydrophobic liquid. The core is more preferably a hydrophobic liquid, such as grease, oil or a mixture thereof. Typical oils may be fish oils, vegetable oils, mineral oils, derivatives thereof or mixtures thereof. The loading substance may comprise a purified or partially purified oily substance such as a fatty acid, a triglyceride or a mixture thereof. Omega-3 fatty acids, such as $\alpha$-linolenic acid (18:3n3), octadecatetraenoic acid (18:4n3), eicosapentaenoic acid (20:5n3) (EPA) and docosahexaenoic acid (22:6n3) (DHA), and derivatives thereof and mixtures thereof, are preferred. Many types of derivatives are well known to one skilled in the art. Examples of suitable derivatives are esters, such as phytosterol esters, branched or unbranched $C_1$-$C_{30}$ alkyl esters, branched or unbranched $C_2$-

$C_{30}$ alkenyl esters or branched or unbranched $C_3$-$C_{30}$ cycloalkyl esters, in particular phytosterol esters and $C_1$-$C_6$ alkyl esters. Preferred sources of oils are oils derived from aquatic organisms (e.g. anchovies, capelin, Atlantic cod, Atlantic herring, Atlantic mackerel, Atlantic menhaden, salmonids, sardines, shark, tuna, etc) and plants (e.g. flax, vegetables, algae, etc).

While the core may or may not be a biologically active substance such as a tocopherol, antioxidant or vitamin, the microcapsules of the present invention are particularly suited for biologically active substances, for example, drugs, nutritional supplements, flavours, antioxidants or mixtures thereof.

Shell Material

Coacervation is a phase separation phenomenon, in which a homogenous polymer solution is converted into two phases. One is a polymer-rich phase, called a coacervate. The other is a polymer-poor phase, i.e., solvent. Complex coacervation is caused by the interaction of two oppositely charged polymers.

Preferably, a positively charged polymer component "A" interacts with a negatively charged polymer component "B". For example, positively charged type A gelatine ("component A") forms complex coacervates with negatively charged polyphosphate ("component B"). Other systems that have been studied are gelatine/gum Acacia, gelatine/pectin, gelatine/carboxymethyl guar gum and whey protein/gum arabic.

Component A is preferably gelatine type A, chitosan, etc., although other polymers are also contemplated as component A. Component B is preferably gelatine type B, polyphosphate, gum arabic, alginate,

carrageenan, pectin, carboxymethylcellulose, or a mixture thereof.

In addition to the charge density of the two polymer components, complex coacervation depends on other factors such as molecular weight of the polymers and their ratio, ionic strength, pH and temperature of the medium (*J. Microencapsulation, 2003, Vol. 20, No. 2: 203-210*).

The molar ratio of component A:component B that is used depends on the type of components but is typically from 1:5 to 15:1. For example, when gelatine type A and polyphosphate are used as components A and B respectively, the molar ratio of component A:component B is preferably 8:1 to 12:1; when gelatine type A and gelatine type B are used as components A and B respectively, the molar ratio of component A:component B is preferably 2:1 to 1:2; and when gelatine type A and alginate are used as components A and B respectively, the molar ratio of component A:component B is preferably 3:1 to 5:1.

One suitable process of microencapsulation using complex coacervation comprises three steps: 1) dispersing the loading substance into a system of at least one of the polymers for the complex coacervate; 2) forming shells by deposition of coacervates which derive from the polymeric components under controlled conditions of temperature, pH, concentration of colloids, mixing speed etc.; and 3) hardening of the shells by crosslinking of the coacervates deposited on microcapsules (*Ullmann's Encyclopedia of Industrial Chemistry 6$^{th}$ edition. 2001, Vol. A16. pp. 575-588*).

Any shells that do not comprise complex coacervates may be formed of any material that can form an additional shell around the microcapsule. The additional

shell material typically comprises at least one polymer component. Examples of polymer components include, but are not limited to, proteins, e.g. gelatines, soy proteins, whey proteins, and milk proteins, polyphosphate, polysaccharides and mixtures thereof. Preferred polymer components are gelatine A, gelatine B, polyphosphate, gum arabic, alginate, chitosan, carrageenan, pectin, cellulose or derivatives of cellulose such as carboxymethylcellulose (CMC) or a mixture thereof. A particularly preferred form of gelatine type A has a Bloom strength of 50-350, more preferably a Bloom strength of about 275.

The shell material can also comprise lipids, such as waxes, fatty acids and oils, etc. to provide desired functionalities. The incorporation of lipids into the shell material improves the impermeability of the shell to water and oxygen. A preferred lipid for this purpose is beeswax. These lipids may be in solid, semi-solid or liquid form.

Processing Aids

Processing aids may be included in the shell material. Processing aids may be used for a variety of reasons. For example, they may be used to promote agglomeration of primary microcapsules when forming multi-core microcapsules, control microcapsule size and shape and/or to act as an antioxidant. Antioxidant properties are useful both during the process (e.g. during coacervation and/or spray drying) and in the microcapsules after they are formed (e.g. to extend shelf-life of loading substances which are readily oxidized, etc). Preferably a small number of processing aids that perform a large number of functions are used. For example, ascorbic acid or a salt thereof may be used to promote agglomeration of the primary microcapsules, to control microcapsule size and shape and to act as an antioxidant. The ascorbic acid or salt thereof is

preferably used in an amount of about 100 ppm to about 10,000 ppm, more preferably about 1000 ppm to about 5000 ppm relative to the batch size (i.e., the total weight). A salt of ascorbic acid, such as sodium or potassium ascorbate, is particularly preferred in this capacity. Other processing aids include, without limitation, buffering acids and/or their salts such as phosphoric acid, acetic acid, citric acid, and the like.

Structure of Microcapsules

In one embodiment, microcapsules of the invention have a structure generally as depicted in Figure 2. Figure 2 depicts a multi-core microcapsule prepared according to a multi-step process of the invention. Primary microcapsules comprise cores 18 (i.e. the loading substance) surrounded by first shells 20. The primary microcapsules agglomerate and the space 22 between them is usually at least partly filled by additional shell material of same composition as first shell 20, although there may be voids between some of the primary microcapsules. The agglomeration of primary microcapsules is surrounded by a second shell 24.

Multi-core microcapsules comprising second shell 24 may be prepared according to the processes described herein and exemplified in the examples or by generally the same techniques that are described in Applicant's co-pending United States Patent Application No. 10/120,621 filed April 11, 2002, corresponding to International Application No. PCT/CA2003/000520 filed April 8, 2003, the disclosures of both of which are incorporated herein by reference. These multi-core microcapsules are particularly useful because the foam-like structure of primary microcapsules, supported by additional shell material in space 22 and surrounded by second shell 24 is an extremely strong, rupture-resistant structure that has a high payload i.e. the ratio of the

total mass of the cores to the total mass of the multi-core microcapsule is very high, e.g. at least 50, 55, 60, 65, 70, 75, 80, 85, 90% or higher. This is called a "one-step" process when shells 20 and 24 are of the same composition and formed in a single step. When shells 20 and 24 are of different composition, the process involves two steps.

Commercially available multicore microcapsules may also be used as starting materials. An example is the Driphorm™ Hi-DHA™ microencapsulated tuna oil, manufactured by Nu-Mega Ingredients Pty. Ltd., Queensland, AU.

In accordance with the invention, a three-step process takes place when a third shell 26 is formed on the multi-core microcapsule. Third shell 26 further strengthens the microcapsule and can be advantageously used to provide a shell having properties different from those of shell 24. For instance, different polymer components can be incorporated into third shell 26. In addition, or alternatively, lipids may be incorporated into shell 26 to increase moisture or oxygen impermeability or the like. These properties might instead be incorporated into second shell 24 rather than third shell 26 (or also into second shell 24 as well as into third shell 26), depending on the requirements for a particular purpose. Additional shells, not shown in Figure 2, may be formed around third shell 26, by the methods and techniques of the invention. For instance, N additional shells could be added, wherein N is an integer from 1 to 20.

At least one of shells 20, 24 and 26 and of any additional shells comprises a complex coacervate, as described above. Preferably, at least two of the shells comprise a complex coacervate. Even more preferably, all of the shells comprise a complex coacervate. For instance, the following shells may comprise complex coacervates: (a) shell

20; (b) shell 24; (c) shell 26; (d) shells 20 and 24; (e) shells 20 and 26; (f) shells 24 and 26; or (g) shells 20, 24 and 26. Additional shells also preferably comprise a complex coacervate.

Referring again to Figure 2, the primary microcapsules (i.e. cores 18 surrounded by first shells 20) typically have an average diameter of about 40 nm to about 10 μm, more particularly from about 0.1 μm to about 5 μm, even more particularly an average diameter of about 1 - 2 μm. The finished multi-core microcapsule, i.e. including third shell 26, usually has an average diameter from about 1 μm to about 2000 μm, more typically from about 20 μm to about 1000 μm, more particularly from about 20 μm to about 100 μm and even more particularly from about 50 μm to about 100 μm.

In Figure 2, second shell 24 and third shell 26 are depicted as discrete layers. This will be the case if the shells are formed of the different shell materials. In that case, even if they do not differ in appearance, they will have a different composition and can be represented as discrete, distinct layers. But if second shell 24 and third shell 26 are formed of the same shell material, they may, as shown in Figure 3, merge to form a single, continuous layer, having the combined thickness of second shell 24 and third shell 26. As shown in Figure 3, when the second and third shells are of the same composition, there may be no discrete boundary separating them. This would be true also in microcapsules of the invention having fourth or additional shells that are of the same composition as the preceding shell.

The invention is also useful in the preparation of single-core microcapsules having multiple shells. Single-core microcapsules useful as starting materials are

commercially available. Examples include microencapsulated flavours by Givaudan Flavors Corp., Cincinnati, Ohio, USA, and microencapsulated minerals and vitamins by Watson Food Co. Inc., West Haven, CT., USA. Alternatively, they can be made by complex coacervation processes as described herein, e.g. by preparing primary microcapsules without a further agglomeration step. Figure 4 depicts a single-core microcapsule having multiple shells in accordance with the invention. Core 18 is surrounded by a first shell 20 and a second shell 24. Additional shells, not shown in Figure 4, may be formed around second shell 24, by the methods and techniques of the invention. For instance, N additional shells could be added, wherein N is an integer from 1 to 20.

As with the multi-core microcapsules, shells 20 and 24 of single-core microcapsules may be of the same or different composition. At least one of shells 20 and 24 and of any additional shells comprises complex coacervates as described above. Preferably, at least two of the shells comprise a complex coacervate. Even more preferably all of the shells comprise a complex coacervate. For instance, the following shells may comprise complex coacervates: (a) shell 20; (b) shell 24; or (c) shells 20 and 24. Additional shells also preferably comprise complex coacervates.

Single-core microcapsules may be as large as multi-core microcapsules. For instance, the exterior diameter of second shell 24 in the single-core microcapsule of Figure 4 may be from about 1 $\mu$m to about 2000$\mu$m. More typically it will be from about 20$\mu$m to about 1000 $\mu$m, more particularly from about 20$\mu$m to about 100 $\mu$m and even more particularly from about 50 $\mu$m to about 100 $\mu$m.

When they are of the same composition, first shell 20 and second shell 24 (and any additional shell) of the single-core multicapsule may merge to form a single

continuous layer as depicted in Figure 5. This may be done in a one-step process.

Processes

Single or multi-core microcapsules to which additional shells may be added by the processes of the invention may be obtained from commercial sources. In a particularly preferred embodiment, multi-core microcapsules prepared in accordance with applicant's co-pending United States Patent Application No. 10/120,621 filed April 11, 2002, corresponding to International Application No. PCT/CA2003/000520 filed April 8, 2003, the disclosures of both of which are incorporated herein by reference, are used. Such microcapsules can be prepared e.g. by a one step process as follows.

An aqueous mixture of a loading substance (i.e. core material) and a polymer component of the shell material is formed. The aqueous mixture may be a mechanical mixture, a suspension or an emulsion. When a liquid loading material is used, particularly a hydrophobic liquid, the aqueous mixture is preferably an emulsion of the loading material and the polymer components.

In a more preferred aspect, a first polymer component is provided in aqueous solution, preferably together with processing aids, such as antioxidants. A loading substance may then be dispersed into the aqueous mixture, for example, by using a homogenizer. If the loading substance is a hydrophobic liquid, an emulsion is formed in which a fraction of the first polymer component begins to deposit around individual droplets of loading substance to begin the formation of primary shells. If the loading substance is a solid particle, a suspension is formed in which a fraction of the first polymer component

begins to deposit around individual particles to begin the formation of primary shells. At this point, another aqueous solution of a second polymer component may be added to the aqueous mixture.

Droplets or particles of the loading substance in the aqueous mixture preferably have an average diameter of less than 100 μm, more preferably less than 50 μm, even more preferably less than 25 μm. Droplets or particles of the loading substance having an average diameter less than 10 μm or less than 5 μm or less than 3 μm or less than 1 μm may be used. Particle size may be measured using any typical equipment known in the art, for example, a Coulter™ LS230 Particle Size Analyzer, Miami, Florida, USA.

The amount of the polymer components of the shell material provided in the aqueous mixture is typically sufficient to form both the primary and outer shells of microcapsules. Preferably, the loading substance is provided in an amount of from about 1% to about 15% by weight of the aqueous mixture, more preferably from about 3% to about 8% by weight, and even more preferably about 6% by weight.

If a complex coacervate is desired, the pH, temperature, concentration, mixing speed or a combination thereof is then adjusted to accelerate the formation of the primary shells of complex coacervate around the droplets or particles of the loading substance to form primary microcapsules. In the case of multicore microcapsules, agglomeration of the primary microcapsules will take place to form discrete clumps at desired size and shape.

pH is an expression of the concentration of hydrogen ions in solution. Such ions affect the ionization equilibria of the component A and B polymers involved in

complex coacervation and thus the formation of complex coacervates. The pH is adjusted so that the component A polymer will bear a net positive charge and the component B polymer will bear a net negative charge. Hence, the pH adjustment depends on the type of shell material to be used.

For example, when gelatine type A is a polymer component, the gelatine molecules have nearly equal positive and negative charges (i.e. zero net polarity change) at their point of zero charge (pzc) around pH 9-10. Only when the solution pH is lower than the pzc value, will the polymer bear a net positive charge, which interacts with the negatively charged component B (e.g. gum arabic, polyphosphate, alginate, etc.).

In the case of gelatine type A, the pH is preferably adjusted to a value from 3.5-5.0, more preferably from 4.0-5.0. Much outside this range, the gelatine-based complex tends to form gels upon cooling rather than a shell on the microcapsules. If the pH of the mixture starts in the desired range, then little or no pH adjustment is required.

The molar ratio of components A and B is adjusted to favour formation of shells on the microcapsules rather than merely the formation of gel particles in solution. Suitable molar ratios are discussed above under the heading "Shell Material".

The concentration of components A and B in the aqueous mixture may also affect the formation of complex coacervates and can be adjusted accordingly. Typically, the total concentration of components A and B varies from 1% to 20%, preferably 2-10%, and more preferably 3-6% by weight of the aqueous mixture. For instance, when gelatine type A is used as component A, the concentration of gelatine type A is

preferably from 1-15% by weight of the aqueous mixture, more preferably 2-6% by weight and even more preferably 2-4% by weight. Similarly, when polyphosphate is used as component B, its concentration in the aqueous mixture is preferably 0.01- 0.65% by weight of the aqueous mixture, more preferably 0.13 - 0.17% by weight, even more preferably 0.13 - 0.26% by weight.

The initial temperature of the aqueous mixture is preferably set to a value of from about 40°C to about 60°C, more preferably at about 50°C.

Mixing speed influences the deposition of complex coacervates on the surface of microcapsules. If the mixing speed is too low, the aqueous mixture is agitated insufficiently and undesirably large microcapsules may be formed. Conversely, if the mixing speed is too high, high shear forces are generated and prevent shell material from forming on the microcapsules. Instead, gel particles form in the solution. The mixing speed is preferably between 100 and 1500 rpm, more preferably between 400 and 1000 rpm and even more preferably between 600 and 800 rpm. Particular mixing parameters depend on the type of equipment being used. Any of a variety of types of mixing equipment known in the art may be used. Particularly useful is an axial flow impeller, such as Lightnin™ A310 or A510.

At this time, materials for outer shell are added into the mixture, and the aqueous mixture may then be cooled under controlled cooling rate and mixing parameters to permit coating of the primary microcapsules to form outer shells. It is advantageous to control the formation of the outer shell at a temperature above the gel point of the shell material. It is also possible at this stage to further add more polymer components, either of the same kind or a different kind, in order to thicken the outer shell

and/or produce microcapsules having different layers of shells to provide desired functionalities. The temperature is preferably lowered at a rate of about 1°C/10 minutes until it reaches a temperature of from about 5°C to about 10°C, preferably about 5°C. The outer shell encapsulates the primary microcapsules or clumps to form a rigid encapsulated agglomeration of microcapsules.

At this stage, a cross-linker may be added to further increase the rigidity of the microcapsules by cross-linking the shell material in both the outer and primary shells and to make the shells insoluble in both aqueous and non-aqueous (e.g., oil) media. Any suitable cross-linker may be used and the choice of cross-linker depends somewhat on the choice of shell material. Preferred cross-linkers are enzymatic cross-linkers (e.g. transglutaminase), aldehydes (e.g. formaldehyde or gluteraldehyde), tannic acid, alum, organic or inorganic calcium or potassium salt, or a mixture thereof. When the microcapsules are to be used to deliver a biologically active substance to an organism, the cross-linkers are preferably non-toxic or of sufficiently low toxicity. The type and the amount of cross-linker used depend on the type of shell material and may be adjusted to provide more or less structural rigidity as desired. For example, when gelatine type A is used in the shell material, transglutaminase may be conveniently used in an amount of about 0.2% to about 2.0%, preferably about 1.0%, by weight of microcapsule suspension. In general, one skilled in the art may routinely determine the desired amount in any given case by simple experimentation.

At this stage, multi-core microcapsules have been produced. These microcapsules or other microcapsules may then be processed in accordance with the invention to add additional shell layers as described above. Preferably, additional shells are added after the formation of the outer

shell of the microcapsule or before the cross-linking step. More particularly, first and second polymer components of shell material are dissolved in aqueous solution e.g. at 40 to 60°C, more preferably around 50°C. pH may be controlled or adjusted at this stage. The microcapsules previously prepared are then combined with this mixture. Alternatively, the microcapsules may be combined with an aqueous solution of the first polymer component of shell material and then a second aqueous solution of the second polymer component of shell material may be added. pH, temperature, concentration, mixing speed or a combination thereof can then be adjusted as described above so that the polymer components of shell material form a complex coacervate surrounding and coating the microcapsules with an additional shell. As discussed above, processing aids may be incorporated as may be hydrophobic materials such as oils, waxes, resins or fats. The new outer shell may be then cross-linked as described above. These additional steps of forming additional shell layers may be repeated as desired to build up a suitable number of further shells on the microcapsule.

Finally, the microcapsules may be washed with water and/or dried to provide a free-flowing powder. Drying may be accomplished by a number of methods known in the art, such as freeze drying, drying with ethanol or spray drying. Spray drying is a particularly preferred method for drying the microcapsules. Spray drying techniques are disclosed in "Spray Drying Handbook", K. Masters, 5th edition, Longman Scientific Technical UK, 1991, the disclosure of which is hereby incorporated by reference.

Uses

The microcapsules produced by the processes of the present invention may be used to prepare liquids as free-

flowing powders or compressed solids, to store a substance, to separate reactive substances, to reduce toxicity of a substance, to protect a substance against oxidation, to deliver a substance to a specified environment and/or to control the rate of release of a substance. In particular, the microcapsules may be used to deliver a biologically active substance to an organism for nutritional or medical purposes. The biologically active substance may be, for example, a nutritional supplement, a flavour, a drug and/or an enzyme. The organism is preferably a mammal, more preferably a human. Microcapsules containing the biologically active substance may be included, for example, in foods or beverages or in drug delivery systems. Use of the microcapsules of the present invention for formulating a nutritional supplement into human food is particularly preferred.

Microcapsules of the present invention have good rupture strength to help reduce or prevent breaking of the microcapsules during incorporation into food or other formulations. Furthermore, the microcapsules' shells can be formulated to be insoluble in both aqueous and non-aqueous (e.g., oil) media, and help reduce or prevent oxidation and/or deterioration of the loading substance during preparation of the microcapsules, during long-term storage, and/or during incorporation of the microcapsules into a formulation vehicle, for example, into foods, beverages, nutraceutical formulations or pharmaceutical formulations.

The invention will now be further illustrated by the following non-limiting examples.

Examples

Example 1: Multicore microcapsules prepared by one-step process for comparison (both first and second shells having the same composition of gelatine and polyphosphate)

54.5 grams gelatine 275 Bloom type A (isoelectric point of about 9) was mixed with 600 grams of deionized water containing 0.5% sodium ascorbate under agitation at 50°C until completely dissolved. 5.45 grams of sodium polyphosphate was dissolved in 104 grams of deionized water containing 0.5% sodium ascorbate. 90 grams of a fish oil concentrate containing 30% eicosapentaenoic acid ethyl ester (EPA) and 20% docosahexaenoic acid ethyl ester (DHA) (available from Ocean Nutrition Canada Ltd.) was dispersed with 1.0% of an antioxidant (mixed natural tocopherols) into the gelatine solution with a high speed Polytron™ homogenizer at 5,500 rpm for 6 minutes. An oil-in-water emulsion was formed. The oil droplet size had a narrow distribution with an average size of about 1 μm measured by Coulter™ LS230 Particle Size Analyzer. The emulsion was diluted with 700 grams of deionized water containing 0.5% sodium ascorbate at 50°C. The sodium polyphosphate solution was then added into the emulsion and mixed with a Lightnin™ agitator at 600 rpm. The pH was then adjusted to 4.5 with a 10% aqueous acetic acid solution. During pH adjustment and the cooling step that followed pH adjustment, a coacervate formed from the gelatine and polyphosphate coated onto the oil droplets to form primary microcapsules. Cooling was carried out to above the gel point of the gelatine and polyphosphate and the primary microcapsules started to agglomerate to form lumps under agitation. Upon further cooling of the mixture, polymer remaining in the aqueous phase further coated the lumps of primary microcapsules to form an encapsulated agglomeration of microcapsules having

an outer shell and having an average size of 50 μm. Once the temperature had been cooled to 5°C, 2.7 grams of 50% gluteraldehyde was added into the mixture to further strengthen the shell. The mixture was then warmed to room temperature and kept stirring for 12 hours. Finally, the microcapsule suspension was washed with water. The washed suspension was then spray dried to obtain a free-flowing powder. A payload of 62% was obtained.

Example 2: A two-step process with gelatine and polyphosphate in both first and second shells, but having different compositions

Step A: 15.6 grams gelatine 275 Bloom type A (isoelectric point of about 9) was mixed with 172 grams of deionized water containing 0.5% sodium ascorbate under agitation at 50°C until completely dissolved. 1.56 grams of sodium polyphosphate was dissolved in 29.7 grams of deionized water containing 0.5% sodium ascorbate. 69 grams of a fish oil concentrate containing 30% eicosapentaenoic acid ethyl ester (EPA) and 20% docosahexaenoic acid ethyl ester (DHA) (available from Ocean Nutrition Canada Ltd.) was dispersed with 1.0% of an antioxidant (mixed natural tocopherols) into the gelatine solution with a high speed Polytron™ homogenizer at 6,100 rpm for 4 minutes. An oil-in-water emulsion was formed. The oil droplet size had a narrow distribution with an average size of about 1 μm measured by Coulter™ LS230 Particle Size Analyzer. The emulsion was diluted with 319 grams of deionized water containing 0.5% sodium ascorbate at 50°C. The sodium polyphosphate solution was then added into the emulsion and mixed with a Lightnin™ agitator at 600 rpm. The pH was then adjusted to 4.5 with a 10% aqueous phosphoric acid solution. During pH adjustment and the cooling step that followed pH adjustment, a coacervate formed from the gelatine and

polyphosphate coated onto the oil droplets to form primary microcapsules, and then the primary microcapsules started to agglomerate to form lumps under agitation. A payload of 80% was obtained at this step.

Step B: A gelatine solution was prepared by dissolving 41.8 grams of gelatine 275 Bloom type A (isoelectric point of about 9) in 460 grams of deionized water containing 0.5% sodium ascorbate under agitation at 50°C until completely dissolved. A sodium polyphosphate solution was prepared by dissolving 4.18 grams of sodium polyphosphate in 79.5 grams of deionized water containing 0.5% sodium ascorbate. The gelatine and polyphosphate solutions were combined to form a mixture, and pH of the mixture was adjusted to 4.7 with 10% aqueous phosphoric acid.

Step C: The mixture from Step B was added to the mixture with lumps formed in step A. Cooling was carried out under agitation to cause the gelatine and polyphosphate to form coacervates and to coat the lumps formed in Step A to form an outer shell. The microcapsules thus formed had an average size of 60 μm. Once the temperature had been cooled to 5°C, 2.1 grams of 50% gluteraldehyde was added into the mixture to further strengthen the shell. The mixture was then warmed to room temperature and stirred continuously for 12 hours. Finally, the microcapsule suspension was washed with water. The washed suspension was then spray dried to obtain a free-flowing powder. A payload of 59% was obtained.

Example 3: A two-step process having gelatine and alginate in the second shell

Step A: Same as Step A in Example 2.

Step B: A gelatine solution was prepared by dissolving 23.0 grams of gelatine 275 Bloom type A (isoelectric point of about 9) in 371 grams of deionized water under agitation at 50°C until completely dissolved. A sodium alginate (ISP Alginates) solution was prepared by dissolving 3.00 grams of sodium alginate in 503.8 grams of deionized water. The gelatine and sodium alginate solutions were combined to form a mixture. The pH of the mixture was adjusted to 5.00 with 10% aqueous phosphoric acid.

Step C: The mixture from Step B was added to the mixture with lumps formed in step A. Cooling was carried out under agitation to cause gelatine and alginate to form coacervates and coat the lumps formed in Step A to form an outer shell. The microcapsules thus formed had an average size of around 80 μm. Once the temperature had been cooled to 5°C, 2.1 grams of 50% gluteraldehyde was added into the mixture to further strengthen the shell. The mixture was then warmed to room temperature and stirred continuously for 12 hours. Finally, the microcapsule suspension was washed with water. The washed suspension was then spray dried to obtain a free-flowing powder. A payload of 53% was obtained.

Example 4: A three-step process to incorporate wax and alginate in the second shell and alginate in the third shell.

Step A: 20.0 grams gelatine 275 Bloom type A (isoelectric point of about 9) was mixed with 220.1 grams of deionized water containing 0.5% sodium ascorbate under agitation at 50°C until completely dissolved. 2.00 grams of sodium polyphosphate was dissolved in 38.0 grams of deionized water. 88.0 grams of a fish oil concentrate containing 30% eicosapentaenoic acid ethyl ester (EPA) and 20% docosahexaenoic acid ethyl ester (DHA) (available from

Ocean Nutrition Canada Ltd.) was dispersed with 1.0% of an antioxidant (mixed natural tocopherols) into the gelatine solution with a high speed Polytron™ homogenizer at 6,100 rpm for 4 minutes. An oil-in-water emulsion was formed. The oil droplet size had a narrow distribution with an average size of about 1 μm measured by Coulter™ LS230 Particle Size Analyzer. The emulsion was diluted with 408.6 grams of deionized water at 50°C. The sodium polyphosphate solution was then added into the emulsion and mixed with a Lightnin™ agitator at 600 rpm. The pH was then adjusted to 4.5 with a 10% aqueous phosphoric acid solution. During pH adjustment and the cooling step that followed pH adjustment, a coacervate formed from the gelatine and polyphosphate coated onto the oil droplets to form primary microcapsules, and then the primary microcapsules started to agglomerate to form lumps under agitation. A payload of 80% was obtained at this step.

Step B: A gelatine solution was prepared by dissolving 8.6 grams of gelatine 275 Bloom type A (isoelectric point of about 9) in 94.5 grams of deionized water under agitation at 65°C until completely dissolved. 25.8 grams of beeswax melted at 65°C was emulsified in the gelatine solution with a high speed Polytron™ homogenizer at 6,100 rpm for 4 minutes. A wax-in-water emulsion was formed. An alginate solution was prepared by dissolving 2.3 grams of sodium alginate in 192 grams of deionized water was added to the emulsion, and pH of the mixture was adjusted to 4.7 with 10% aqueous phosphoric acid. The mixture was then added into lump mixtures in step A under agitation at 800 rpm, and cooling was carried out to cause the gelatine-alginate-wax composite material to form a coating onto the lumps formed in Step A to form microcapsules. A payload of 60% was obtained at this step.

Step C: A solution was prepared by dissolving 23.1 grams of gelatine and 2.3 grams of sodium alginate in 384.9 grams of deionized water under agitation at 50°C until completely dissolved. pH of the mixture was adjusted to 4.5 with 10% aqueous phosphoric acid, and the mixture was then added into microcapsule mixtures formed in step B under agitation at 800 rpm. Cooling was carried out to cause the gelatine-alginate material to form a coating onto the microcapsules that formed in Step B. Once the temperature had been cooled to 5°C, 1.5 grams of transglutaminase was added into the mixture to cross-link the shell. The mixture was then warmed to room temperature and kept stirring for 12 hours. Finally, the microcapsule suspension was spray dried to obtain a free-flowing powder. A final payload of 52% was obtained.

Example 5: A two-step process of multicore microcapsules having wax and alginate in the second shell.

Step A: 13.0 grams of gelatine 275 Bloom type A (isoelectric point of about 9) was mixed with 143.0 grams of deionized water containing 0.5% sodium ascorbate under agitation at 50°C until completely dissolved. 1.3 grams of sodium polyphosphate was dissolved in 24.7 grams of deionized water. 57.2 grams of fish oil containing 18% eicosapentaenoic acid (EPA) and 12% docosahexaenoic acid (DHA) (available from Ocean Nutrition Canada Ltd.) was dispersed with 1.0% of an antioxidant (mixed natural tocopherols) into the gelatine solution with a high speed Polytron™ homogenizer at 8,000 rpm for 4 minutes. An oil-in-water emulsion was formed. The oil droplet size had a narrow distribution with an average size of about 1 μm measured by Coulter™ LS230 Particle Size Analyzer. The emulsion was diluted with 266.0 grams of deionized water at 50°C. The sodium polyphosphate solution was then added into

the emulsion and mixed with a Lightnin™ agitator at 350 rpm. The pH was then adjusted to 4.4 with a 10% aqueous phosphoric acid solution. During pH adjustment and the cooling step that followed pH adjustment, a coacervate formed from the gelatine and polyphosphate coated onto the oil droplets to form primary microcapsules, and then the primary microcapsules started to agglomerate to form lumps under agitation. A payload of 80% was obtained at this step.

Step B: A gelatine solution was prepared by dissolving 7.05 grams of gelatine 275 Bloom type A (isoelectric point of about 9) in 77.9 grams of deionized water under agitation at 70°C until completely dissolved. 7.05 grams of beeswax melted at 70°C was emulsified in the gelatine solution with a high speed Polytron™ homogenizer at 8,000 rpm for 4 minutes. A wax-in-water emulsion was formed. An alginate solution (45 °C) was prepared by dissolving 7.62 grams of sodium alginate in 630 grams of deionized water was added to the emulsion, and pH of the mixture was adjusted to 5.3 with 10% aqueous phosphoric acid. The mixture was then added into lump mixtures in step A under agitation at 450 rpm followed by adjusting the pH value of the mixture to 4.9, and cooling was carried out to cause the gelatine-alginate-wax composite material to form a coating onto the lumps formed in Step A to form microcapsules. Once the temperature had been lowered to 5°C, 3.8 grams of transglutaminase was added into the mixture to cross-link the shells. The mixture was then warmed up to room temperature and stirred at 600 rpm for 12 hours. Finally, the microcapsule suspension was spray dried to obtain a free-flowing powder. A final payload of 57% was obtained.

Example 6: Evaluation of microcapsules

Images of microcapsules of Examples 1-5 are shown in Figures 6 to Figure 10, respectively. It can be seen clearly that at approximately the same payload (60%) the microcapsules prepared with a two step process (Figure 7) have much thicker outer shells than those prepared with one step process (Figure 6). The microcapsules prepared with a three step process having a composite shell containing lipids (Figure 9) clearly show the lipid droplets incorporated in the second shell and near the agglomerated oil core.

Accelerated oxidative stability in dry state was evaluated by placing the prepared microcapsule powders from each of Examples 1-4 in an oxygen bomb (Oxipres™, MIKROLAB AARHUS A/S, Denmark) with an initial oxygen pressure of 5 bar at a constant temperature of 65°C. When the encapsulated fish oil started to oxidize, the oxygen pressure dropped, and an induction period or time was determined. A longer induction period means that the contents of the microcapsules are better protected towards oxidation.

Induction periods are shown in Table 1. The microcapsules made from a two-step process in accordance with the invention have higher induction period (50-56 hours) than those made from a one-step process (41 hours). This translates to 22.0% to 37.6% increase in oxidative stability.

Table I. Comparison of the microcapsules described in Examples 1-5.

| Example # | Figure # | Description | Loading (%) | Induction period (hr) |
|---|---|---|---|---|
| 1 | 6 | Multicore one-step process for comparison | 62 | 41 |
| 2 | 7 | Two-step process with gelatine and polyphosphate in outer shell | 59 | 50 |
| 3 | 8 | Two-step process with alginate in outer shell | 53 | 55 |
| 4 | 9 | Three-step process incorporating wax and alginate in the second shell and gelatine and polyphosphate in the third shell | 52 | 44 |
| 5 | 10 | Two-step process incorporating wax and alginate in the shell | 57 | 56 |

All publications cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference. The citation of any publication should not be construed as an admission that such publication is prior art.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this specification that certain changes or modifications may be made thereto without departing from the spirit or scope of the appended claims.

CLAIMS:

1. A multi-core microcapsule comprising:

(a) an agglomeration of primary microcapsules, each primary microcapsule comprising a core and a first shell surrounding said core;

(b) a second shell surrounding said agglomeration; and

(c) a third shell surrounding said second shell;

at least one of said first, second and third shells comprising a complex coacervate.

2. The multi-core microcapsule according to claim 1, wherein each of said first, second and third shells comprises a complex coacervate.

3. The multi-core microcapsule according to claim 1, wherein each of said first, second and third shells comprises the same complex coacervate.

4. The multi-core microcapsule according to claim 1, wherein at least one of said first, second and third shells comprises a complex coacervate that is different than a complex coacervate that forms one of the other shells.

5. The multi-core microcapsule according to claim 1, wherein said complex coacervate comprises at least one polymer component selected from the group consisting of: a protein, a polyphosphate, a polysaccharide, gum arabic, alginate, chitosan, carrageenan, pectin, cellulose and cellulose derivatives.

6. The multi-core microcapsule according to claim 5, wherein said protein is selected from the group consisting of gelatine type A, gelatine type B, soy proteins, whey proteins, milk proteins, and combinations thereof.

7. The multi-core microcapsule according to claim 1, wherein at least one of said first, second and third shells comprises a complex coacervate between gelatine A and at least one polymer component selected from the group consisting of gelatine type B, polyphosphate, gum arabic, alginate, chitosan, carrageenan, pectin and carboxymethylcellulose.

8. The multi-core microcapsule according to claim 1, wherein at least one of said first, second and third shells is a complex coacervate between gelatine A and polyphosphate.

9. The multi-core microcapsule according to claim 1, further comprising at least one additional shell surrounding said third shell.

10. The multi-core microcapsule according to claim 9, wherein said at least one additional shell surrounding said third shell comprises a complex coacervate.

11. The multi-core microcapsule according to claim 1, wherein at least one of said first second and third shells comprises an antioxidant.

12. The multi-core microcapsule according to claim 1, wherein at least one of said first, second and third shells comprises one or more hydrophobic components selected from the group consisting of waxes, oils, resins, and fats.

13. The multi-core microcapsule according to claim 1, wherein at least one of said first, second and third shells comprises a complex coacervate that is cross-linked with a cross-linker.

14. The multi-core microcapsule according to claim 1, wherein said cores comprise at least 50% of the total mass of the multi-core microcapsule.

15. The multi-core microcapsule according to claim 1, having an exterior average diameter of from about 1 μm to about 2000 μm, and wherein said first shells have an average diameter of from about 40 nm to about 10 μm.

16. A single-core microcapsule comprising:

(a) a core;

(b) a first shell surrounding said core; and

(c) a second shell surrounding said first shell;

at least one of said first and second shells comprising a complex coacervate.

17. The single-core microcapsule according to claim 16, wherein said first and second shells both comprise a complex coacervate.

18. The single-core microcapsule according to claim 16, wherein said first and second shells comprise the same complex coacervate.

19. The single-core microcapsule according to claim 16, wherein said first and second shells comprise different complex coacervates.

20. The single-core microcapsule according to claim 16, wherein said complex coacervate comprises at least one polymer component selected from the group consisting of: a protein, a polyphosphate, a polysaccharide, gum arabic, alginate, chitosan, carrageenan, pectin, cellulose and cellulose derivatives.

21. The single-core microcapsule according to claim 20, wherein said protein is selected from the group consisting of gelatine type A, gelatine type B, soy

proteins, whey proteins, milk proteins, and combinations thereof.

22. The single-core microcapsule according to claim 16, wherein at least one of said first and second shells comprises a complex coacervate between gelatine A and at least one polymer component selected from the group consisting of gelatine type B, polyphosphate, gum arabic, alginate, chitosan, carrageenan, pectin and carboxymethylcellulose.

23. The single-core microcapsule according to claim 16, wherein at least one of said first and second shells comprises a complex coacervate between gelatine A and polyphosphate.

24. The single-core microcapsule according to claim 16, further comprising at least one additional shell surrounding said second shell.

25. The single-core microcapsule according to claim 24, said at least one additional shell surrounding said second shell comprising a complex coacervate.

26. The single-core microcapsule according to claim 16, wherein at least one of said first and second shells comprises an antioxidant.

27. The single-core microcapsule according to claim 16, wherein at least one of said first and second shells comprises at least one hydrophobic component selected from the group consisting of waxes, oils, resins, and fats.

28. The single-core microcapsule according to claim 16, wherein at least one of said first and second shells comprises a complex coacervate that is cross-linked with a cross-linker.

29. The single-core microcapsule according to claim 16, wherein said core comprise at least 60% of the total mass of the microcapsule.

30. The single-core microcapsule according to claim 16, having an exterior average diameter of from about 1 μm to about 2000 μm.

31. A process for making a microcapsule having a plurality of shells, said process comprising:

(a) providing a microcapsule selected from the group consisting of:

(i) a multi-core microcapsule comprising: an agglomeration of primary microcapsules, each primary microcapsule comprising a core and a first shell surrounding said core; and a second shell surrounding said agglomeration; and

(ii) a single-core microcapsule comprising: a core; and a first shell surrounding said core;

(b) mixing said microcapsule with first and second polymer components of shell material in aqueous solution;

(c) adjusting at least one of pH, temperature, concentration and mixing speed to form shell material comprising said first and second polymer components, said shell material forming an additional shell enveloping said microcapsule;

wherein at least one of said first shell, said second shell and said additional shell comprises a complex coacervate.

32. The process according to claim 31, wherein all of said shells comprise a complex coacervate.

33. The process according to claim 31 wherein, in step (b), said microcapsule is mixed with an aqueous solution comprising both said first and second polymer components of shell material.

34. The process according to claim 31 wherein, in step (b), said microcapsule is first mixed with an aqueous solution comprising said first polymer component of shell material and the resulting mixture is then mixed with a second aqueous solution comprising said second polymer component of shell material.

35. The process according to claim 31, comprising the further steps of:

(d) mixing the microcapsule obtained in step (c) with third and fourth polymer components of shell material in aqueous solution;

(e) adjusting at least one of pH, temperature, concentration and mixing speed to form shell material comprising said third and fourth polymer components, said shell material forming a further shell enveloping said microcapsule.

36. The process according to claim 35, wherein said further shell comprises a complex coacervate.

37. The process according to claim 35, wherein said third and fourth polymer components of shell material are the same as said first and second polymer components of shell material.

38. The process according to claim 35, wherein said third and fourth polymer components of shell material are not the same as said first and second polymer components of shell material.

39. The process according to claim 31, wherein said first and second polymer components are selected from the group consisting of: a protein, a polyphosphate, a polysaccharide, gum arabic, alginate, chitosan, carrageenan, pectin, cellulose and cellulose derivatives.

40. The process according to claim 39, wherein said protein is selected from the group consisting of gelatine type A, gelatine type B, soy proteins, whey proteins, milk proteins, and combinations thereof.

41. The process according to claim 31, wherein said first polymer component comprises gelatine type A and said second polymer component comprises gelatine type B, a polyphosphate, gum arabic, alginate, chitosan, carrageenan, pectin or carboxymethylcellulose.

42. The process according to claim 31, wherein step (b) further comprises mixing said microcapsule with an antioxidant.

43. The process according to claim 31, comprising the further step of cross-linking said additional shell with a cross-linker.

44. The process according to claim 31, wherein step (b) further comprises mixing said microcapsule with at least one hydrophobic component selected from the group consisting of waxes, oils, resins and fats.

45. The process according to claim 35, comprising repeating steps (d) and (e) from 1 to 20 times to add at least one additional shell to said microcapsule.

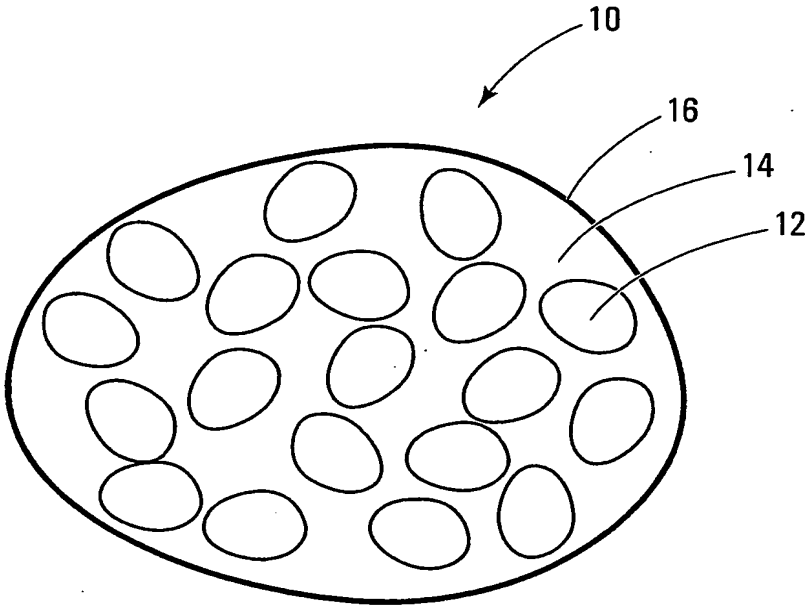



**FIG. 1**
*(PRIOR ART)*

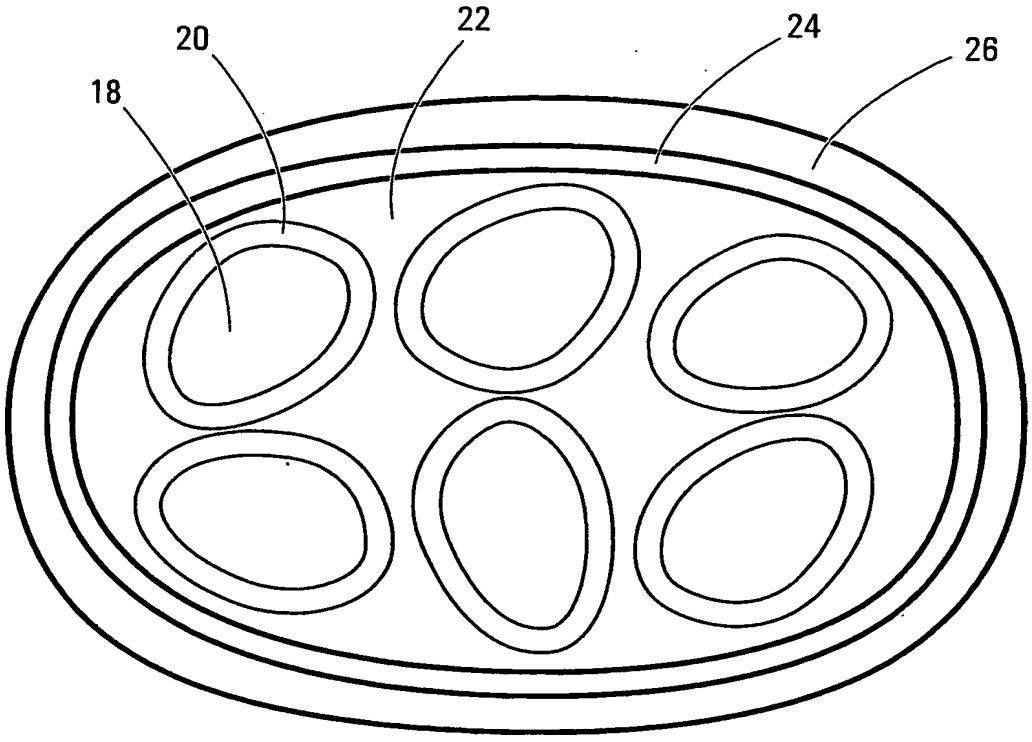



FIG. 2

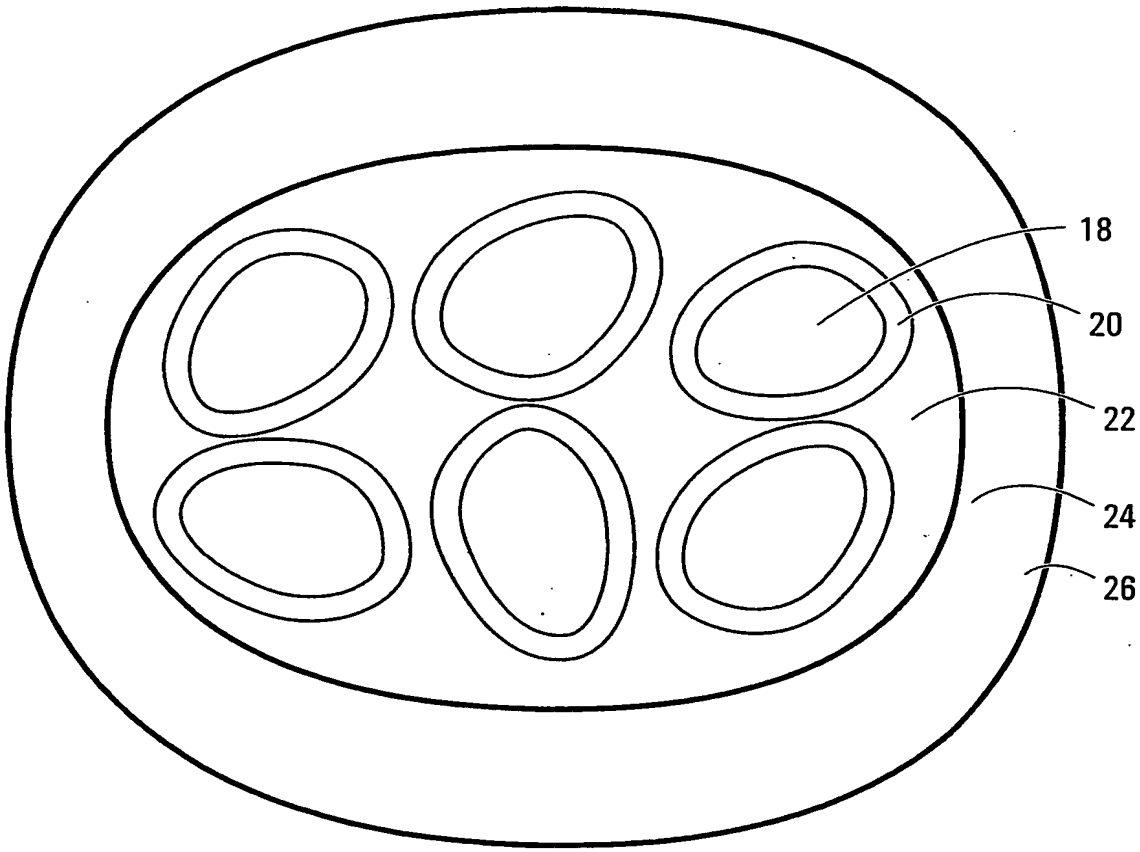



FIG. 3

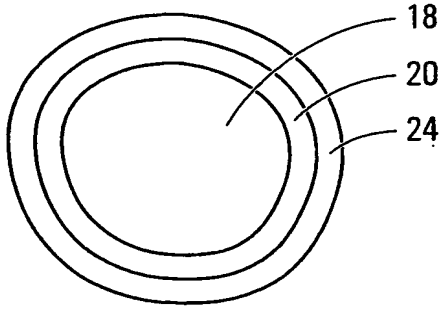



FIG. 4

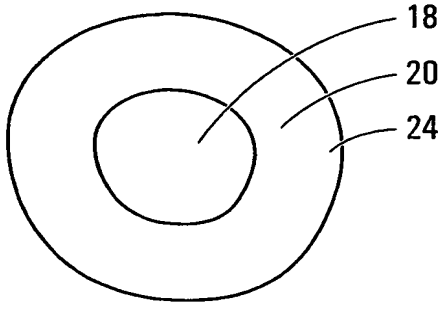



FIG. 5

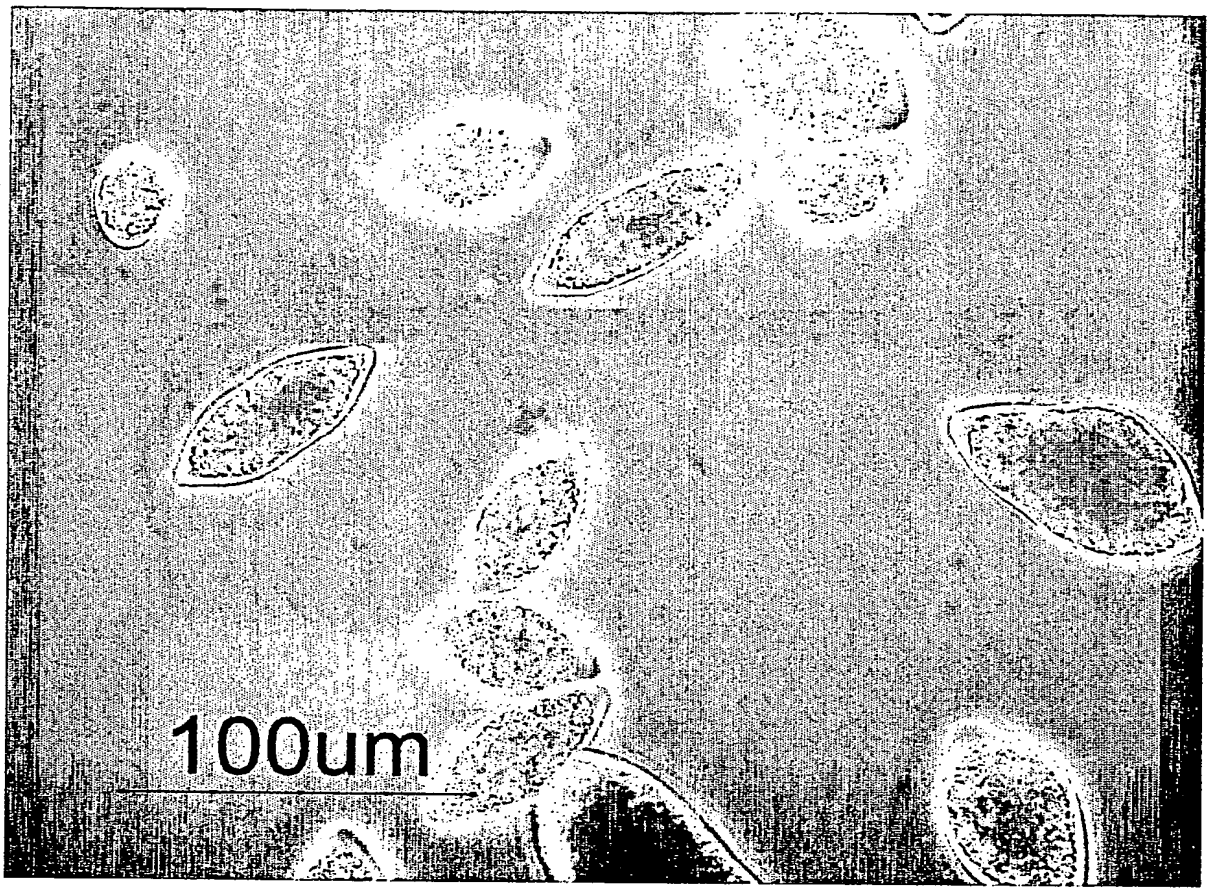



FIG. 6

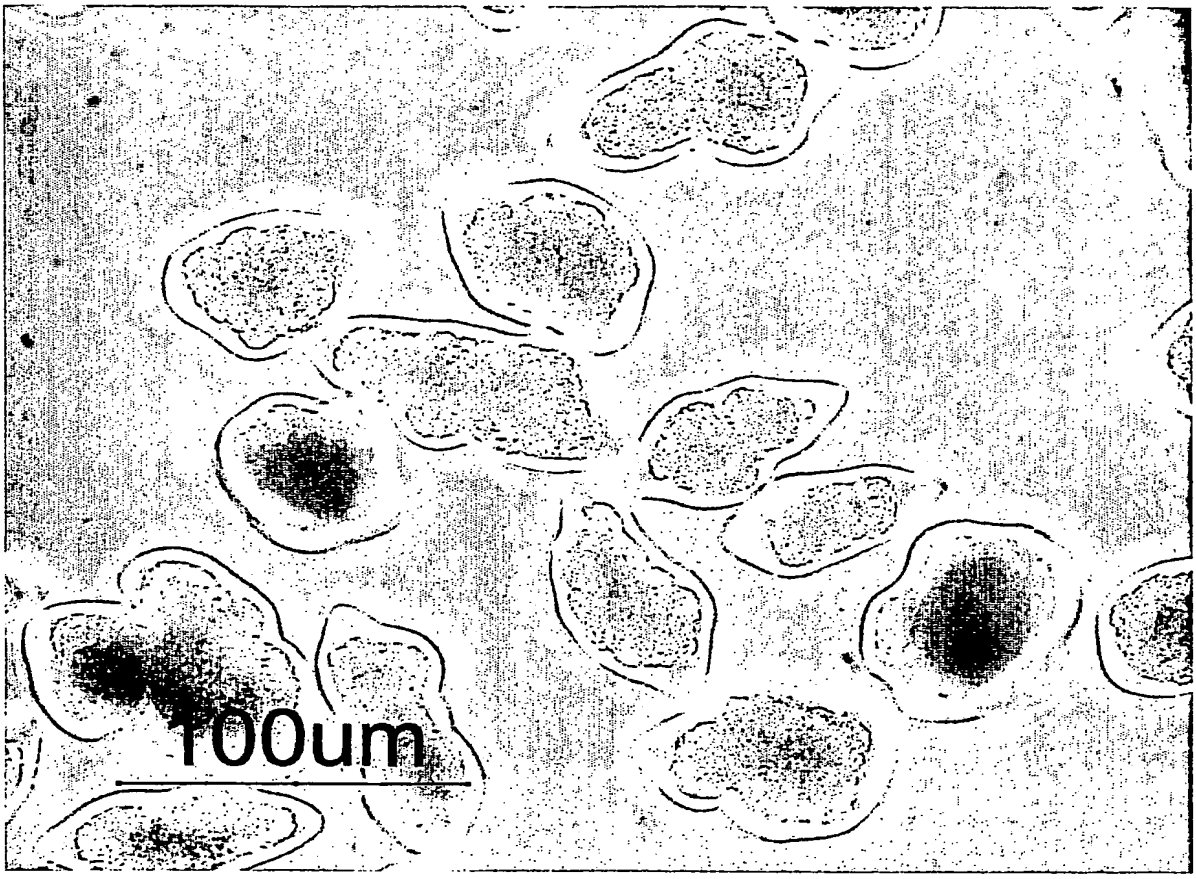



FIG. 7

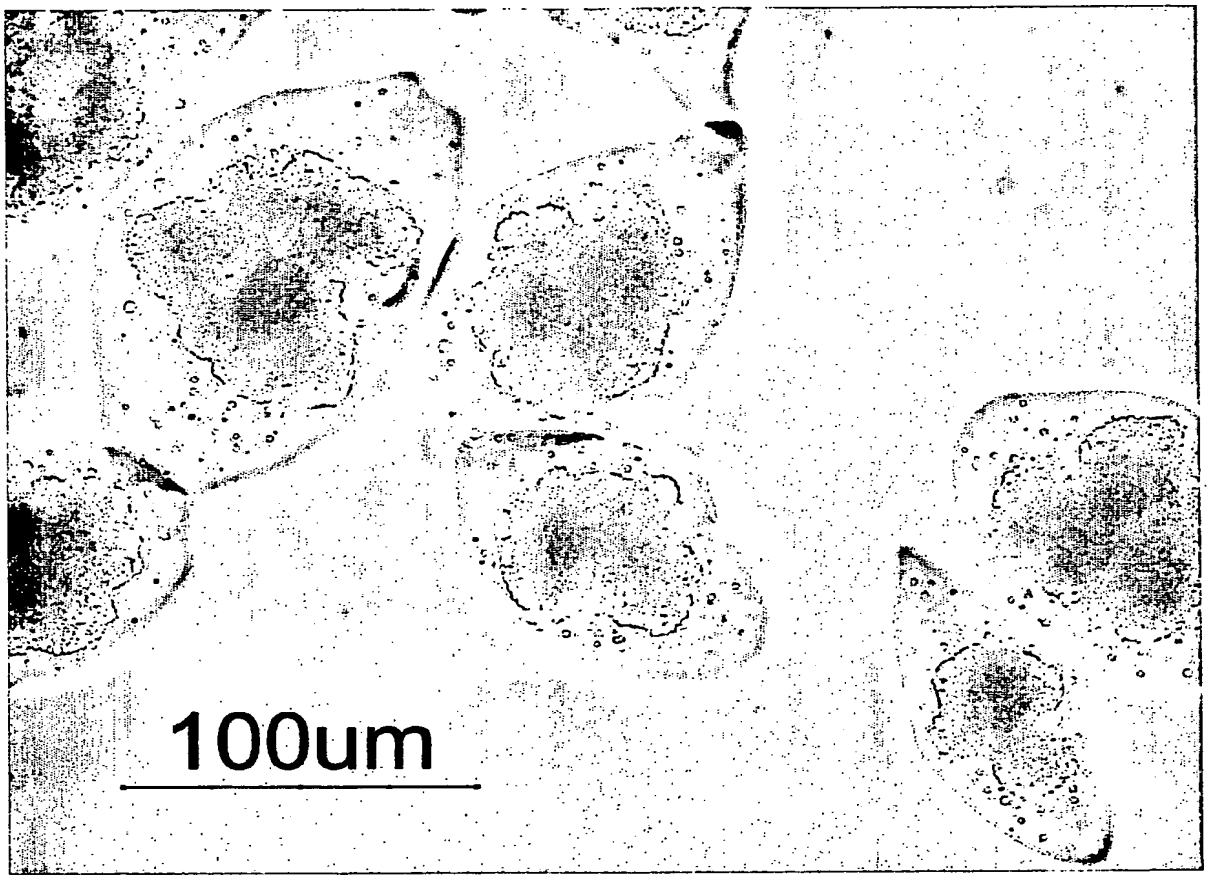



FIG. 8

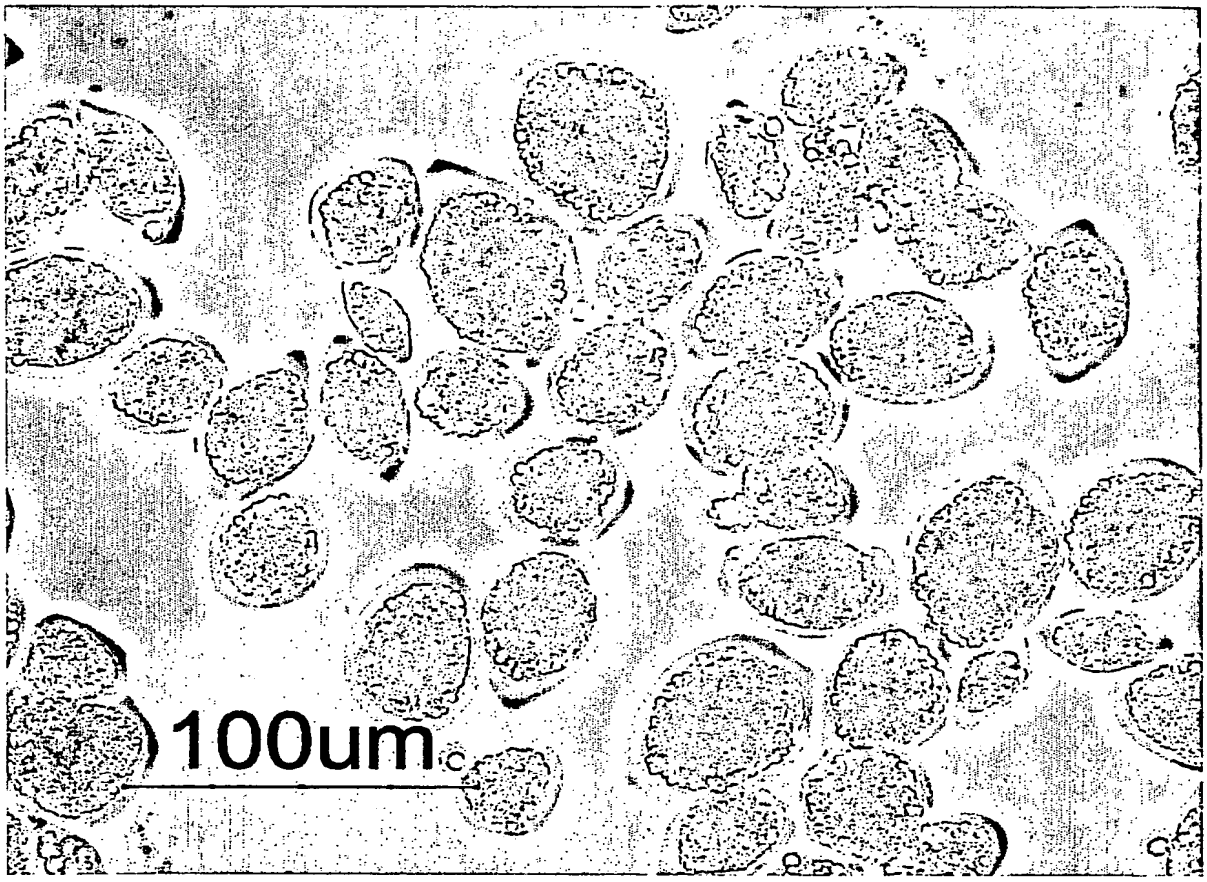



FIG. 9

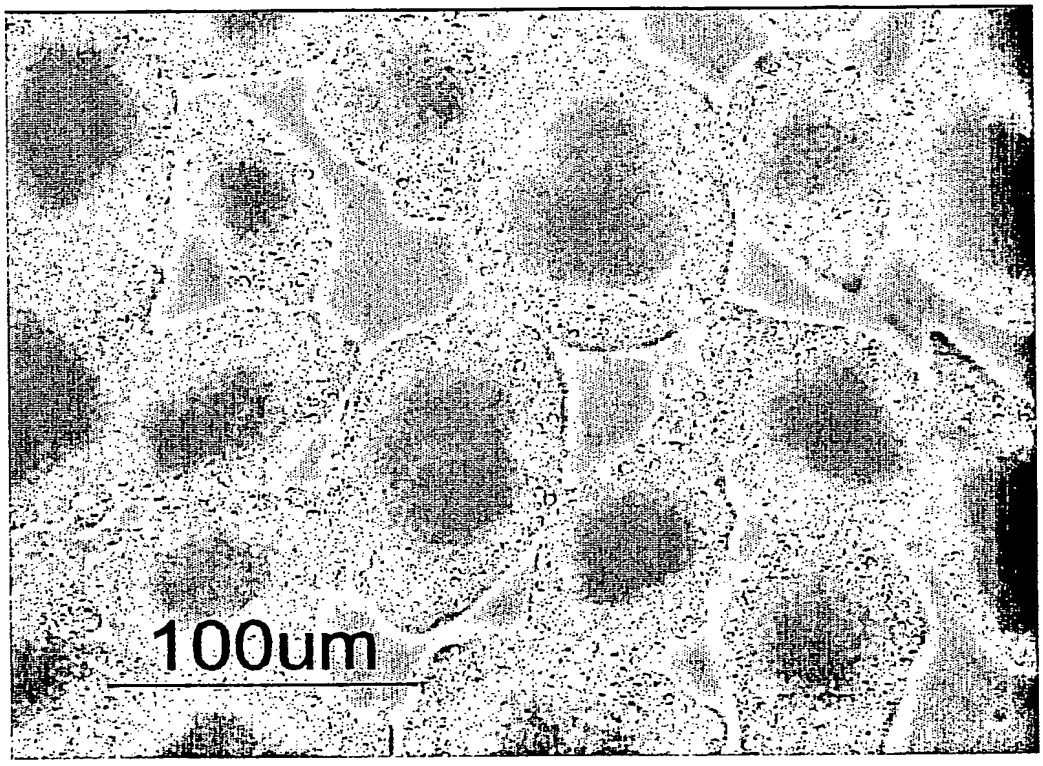



FIG. 10

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 03/01699

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/16 A23L1/22

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | WO 97/13416 A (PORZIO MICHAEL A ;MADSEN MICHAEL G (US)) 17 April 1997 (1997-04-17) page 29 -page 30; example 5 | 1-45 |
| A | EP 0 416 575 A (MEDAPHORE INC) 13 March 1991 (1991-03-13) example 8 | 1-45 |

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 27 April 2004 | 06/05/2004 |

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BNSDOCID: <WO_____2004041251A1_I_>

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